Red Blood Cell dispersion in 100 µm glass capillaries: the temperature effect

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Abstract— The rheological behaviour of the red blood cells (RBCs) flowing in microvessels and microchannels depend on several effects, such as hematocrit (Hct), geometry, and temperature. Previous *in vitro* studies have measured the Hct effect on the radial dispersion (D_{yy}) at both diluted and concentrated suspensions of RBCs. However, according to our knowledge the effect of the temperature on RBC D_{yy} was never studied. Hence, the main purpose of the present work is to investigate the effect of the temperature on the RBC D_{yy}. *In vitro* human blood was pumped through a 100 µm glass capillary and by using a confocal micro-PTV system the RBC D_{yy} was calculated at two different temperatures, i.e., 25ºC and 37ºC.

Keywords— Radial dispersion, red blood cells, temperature, confocal micro-PTV, microcirculation.

I. Introduction

Generally, temperature may play an important role in several phenomena happening in microcirculation. Several studies in the deformation of cells have observed discrepancies between room and body temperature. Recently Lima and his colleagues measured the red blood cells (RBCs) radial dispersion (D_{yy}) in both glass [1, 2] and polydimensiloxane (PDMS) [3]microchannels by using a confocal micro-PTV system. By comparing Lima et al. results with the measurements performed by Goldsmith and Marlow [4] we have observed several quantitative deviations between both experimental results. One possible reason for observed discrepancies may due to the different temperatures used in the two cases, i.e., Lima et al. used body temperatures (~37ºC) whereas Goldsmith and Marlow used room temperatures. Hence, the main objective of the present study is to clarify the effect of the temperature on the RBC D_{yy}. The experiments were performed in the middle of 100 µm glass capillaries at temperatures of 25ºC and 37ºC. The RBC D_{yy} was calculated by using a confocal micro-PTV system.

II. Materials & Methods

Working fluids and Microchannel

The working fluid used in the present study was Dextran 40 (Dx-40; Otsuka Medicine, Tokyo, Japan) containing 12 ± 2% (12Hct) of human RBCs. The Hcts corresponded to the feed reservoir Hct and were measured using a hematocrit centrifuge (Kubota 3220; Kubota Corp., Osaka, Japan) immediately before each experiment. The RBCs were labeled with a lipophilic carbocyanime derivative, chloromethylbenzamido (CM-Dil, C-7000, Molecular Probes, Eugene, OR, USA). A detailed description about the procedure for labeling the human RBCs can be found elsewhere [2].

In this study, we used a 100-µm circular borosilicate glass microchannel fabricated by Vitrocom (Mountain Lakes, NJ, USA). The microchannel was mounted on a slide glass with a thickness of 80 ± 20 µm and was immersed in glycerol to minimize the refraction from the walls.

Experimental setup

The confocal micro-PIV system used in this study consists of an inverted microscope (IX71; Olympus, Japan) combined with a confocal scanning unit (CSU22; Yokogawa, Japan), a diode-pumped solid-state (DPSS) laser (Laser Quantum, UK) with an excitation wavelength of 532 nm and a high-speed camera (Phantom v7.1; Vision Research, USA) (see Fig.1). The microchannels were placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific, USA) a pressure-driven flow was kept constant (Re ~ 0.008). Additionally, by using a thermo plate controller (Tokai Hit) it was possible to apply different temperatures to surrounding environment, i.e., 25ºC±1 and 37ºC±1. More detailed information about this system can be found elsewhere [2, 5-7].

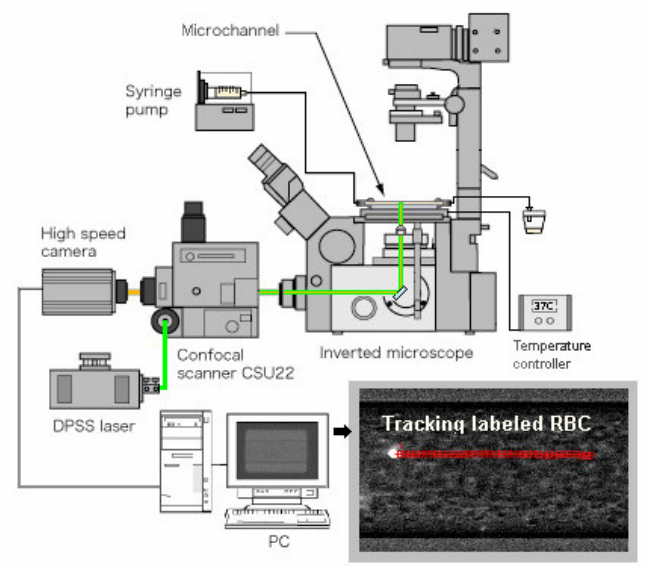

Fig. 1 Confocal micro-PTV experimental set-up (adapted from PhD).

Tracking RBC trajectory

The laser beam was illuminated from below the microscope stage through a dry 40× objective lens with a numerical aperture (NA) equal to 0.9. The confocal images were captured in middle of the capillary with a resolution of 640×480 pixel at a rate of 100 frames/s with an exposure time of 9.4 ms. A manual tracking plugin (MTrackJ) [8] of an image analysis software (Image J, NIH) [9] was used to track the label RBCs. By using MTrackJ plugin, the bright centroid of the selected RBC was automatically computed through successive images so that it was possible to track accurately the labeled RBCs even when two cells were close together After obtaining series of x and y positions, data were exported for the determination of physical dispersion coefficient at different temperatures. Figures 2 and 3 show the trajectories of labeled RBC through a 100-µm circular glass microchannel for a temperature of 25ºC and 37ºC, respectively.

RBC radial dispersion

The RBC trajectories were analysed by using a radial dispersion coefficient (D_{yy}) given by :

$$D_{yy}(t) = \frac{1}{N}\sum_{i=1}^{N}\frac{\left\langle \left(R_{i,y}(t) - R_{i,y}(0)\right)^2 \right\rangle}{2t} \qquad (1)$$

where $R_{i,y}$ and t are the radial displacement and time respectively.

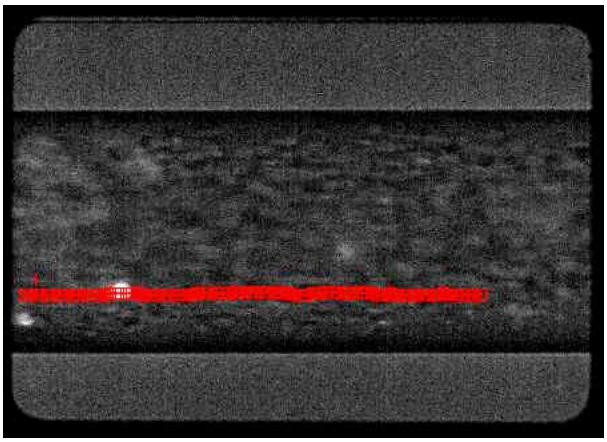

Fig. 2 Radial displacement of labeled RBC in 100-µm glass microchannel at 12%Hct and a surrounding temperature of 25ºC. The image contains both halogen and laser light, that enables to see both labeled and non-labeled RBCs.

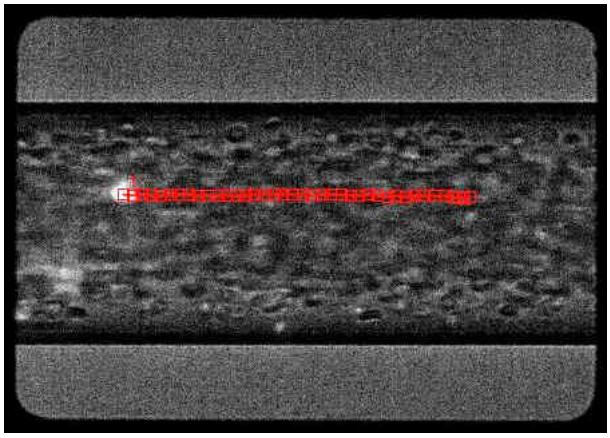

Fig. 3 Radial displacement of labeled RBC in 100-µm glass microchannel at 12%Hct and a surrounding temperature of 37ºC. The non-labeled RBCs are observed as dark-grey rings, whereas labeled RBCs are observed as bright rings.

III. Results & Discussion

The present study aims to obtain further insights on effect of temperature on the RBCs flow behavior in glass capillaries. By using a confocal PTV system, the paths of 18 labeled RBCs were measured in the centre plane of a 100 µm glass capillary. Figures 4 and 5 show typical halogen and confocal image recorded in the middle plane of a 100 µm glass capillary with 12% Hct at a temperature of 25ºC and 37ºC, respectively.

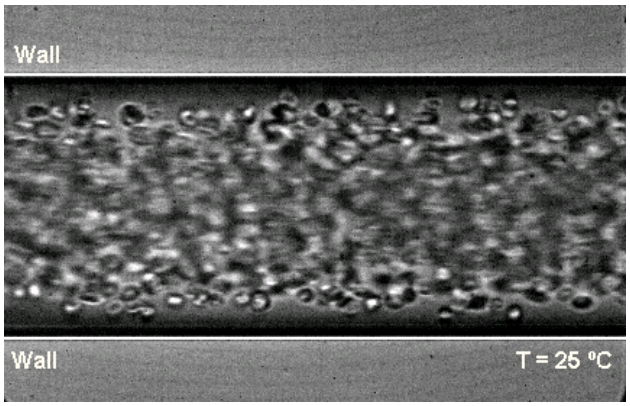

Fig. 4 Halogen image in the middle plane of a 100µm glass capillary with 15% Hct, Re = 0.008 and surrounding temperature of 25ºC.

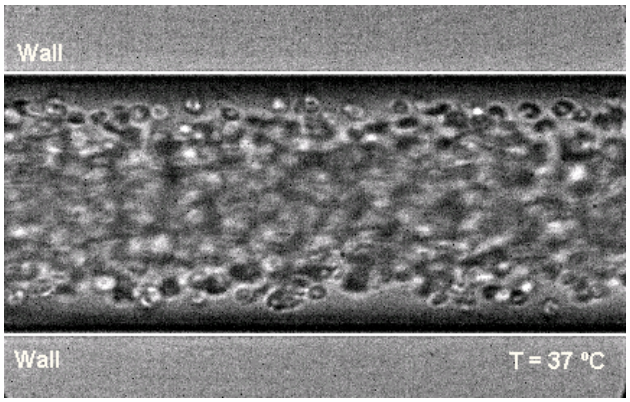

Fig. 5 Halogen image in the middle plane of a 100µm glass capillary with 15% Hct, Re = 0.008 and a surrounding temperature of 37ºC.

By measuring the radial displacement of labeled RBCs flowing through a 100µm capillary for a known time interval, it was possible to calculate the correspondent dispersion coefficient (D_{yy}). By using a manual tracking software eighteen individual labeled RBCs were selected and tracked for each temperature, i.e. 25ºC and 37ºC. Figure 6 shows the RBC averaged dispersion coefficient at the middle plane (D_{yy}) for two different temperatures (25ºC and 37ºC) whereas Figure 7 shows the RBC averaged D_{yy} for two different Hcts (3% Hct and 24% Hct) obtained with a temperature ~37ºC.

A previous study performed by Lima et al. [1] has determined the effect of the Hct (from 3% to 35%) on the radial displacements of labeled RBC. The present work extends their investigations by varying the surrounding temperature and as a result measuring the effect of the temperature on the RBC radial dispersion coefficient. However the preliminary D_{yy} results from Figure 6 does not show any significant difference between both temperatures, especially for values of time bigger than 0.1s where D_{yy} seems to tend to a constant value of approximately 1×10^{-8} cm^2s^{-1}.

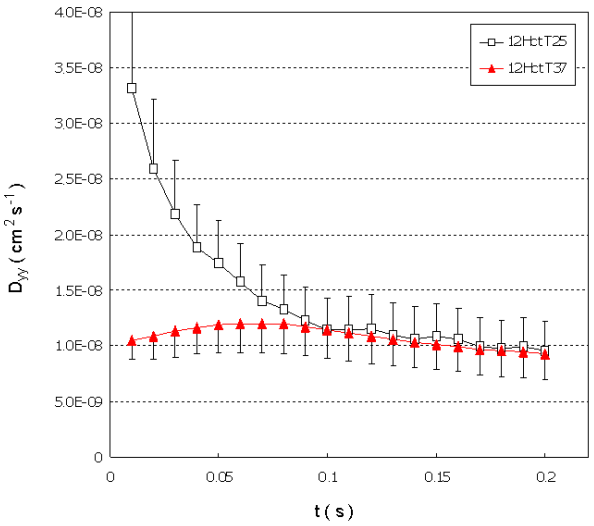

Fig. 6 RBC dispersion coefficient (D_{yy}) at the middle plane for a temperature of 25ºC (12HctT25) and 37ºC (12HctT37). The Hct was12%Hct and the Re was ~0.008.

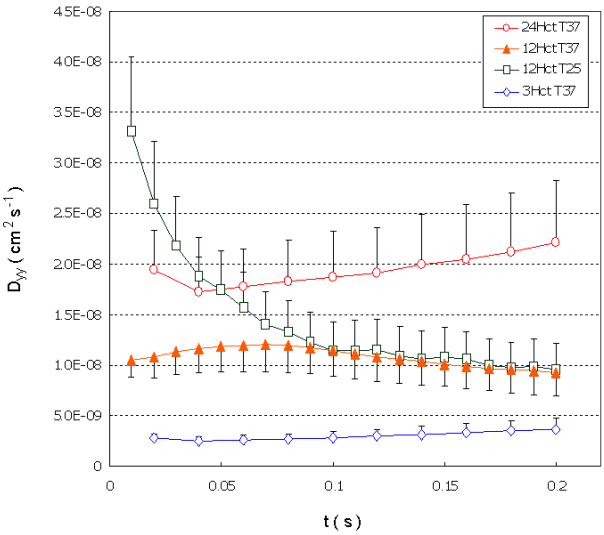

Fig. 7 RBC D_{yy} for a Hct of 12% and a temperature of 37ºC (12HctT37) and 25ºC (12HctT25). Also shown the RBC D_{yy} for a temperature of 37ºC and 3% Hct (3HctT37) and 24% Hct (24HctT37). The Re for the latter results was ~0.005 [1].

Figure 7 shows the comparison between the present results (12%Hct, T = 25ºC & T = 37ºC) and data obtained by Lima et al. (T = 37ºC, 3%Hct & 24%Hct) [1]. As it can be seen, the present results are consistent with those in the literature, which D_{yy} tends to increase with the Hct.

IV. Conclusion & future directions

The main purpose of the present work is to investigate the effect of the temperature on the RBC radial dispersion coefficient (D_{yy}). Although we have observed several qualitative discrepancies related to both translational and rotational motion, the preliminary quantitative results suggest that the temperature does not affect significantly the RBC D_{yy}.

The results obtained from the presented study were determined with eighteen labeled RBCs performed at two different temperatures. In the near future, we expect to measure a bigger amount of RBC´s trajectories for a more number of temperatures.

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